IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

KAI SCHIEMANN et al. Group Art Unit: 1624

Serial No.: 10/551,997 Examiner: J. H. MURRAY

Filed: October 4, 2005

For: CHROMENONE INDOLES

DECLARATION UNDER 37 C.F.R. § 1.132

Honorable Commissionar of
Patents and Trademarks
Washington, D.C. 20231

SIR:

Christoph van Amsterdam, being duly warned, deposes and says:

I am a citizen of the Federal Republic of Germany residing at Darmstadt, Germany;

I am a veterinarian by training and experience;

The degree 'Veterinarian' (at least equivalent to D.V.M.) was bestowed on me by the University of Veterinary Medicine Hannover, Germany; in 1990 and the degree Dr. med. vet. by the University of Veterinary Medicine Hannover, Germany in 1992.

Since June 1995 I have been employed as a pharmacologist in the CNS-Department of Merck KGaA, Darmstadt, Germany.

I am author or co-author of numerous papers and patents in the fields of pharmacology, biochemistry and the development of drugs.

Furthermore, I am familiar with the present application Ser. No. 10/551,997.

I have carried out, or supervised experiments for testing 5-HT, namely 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C} antagonistic properties according to the methods described within the genus claimed in the pending application.

The tested chromenonindole derivatives of the invention act in a 5-HT like fashion and hence are potent and effective 5-HT_{1A} receptor agonists.

Pharmacological Report

Agonism and antagonism at the 5-HT_{1D} receptor can be assessed measuring labelled 5-HT release from guinea pig cortical slices as described by Matzen et al. (Matzen et al., J Med Chem 43, 1149, 2000). As for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, agonistic as well as antagonistic activity can be determined using GTPgammaS binding method. This method is carried out as described in Heinrich et al. (Heinrich et al., Biorg Med Chem 12, 4843, 2004), Bartoszyk et al. (Bartoszyk et al., Eur J Pharmacol 473, 229, 2003) and Pullar et al. (Pullar et al., Eur J Pharmacol 432, 9, 2001).

According to these prior art descriptions, the Applicant has carried out the following experimental procedure for assessing the agonistic activity of the compounds of the invention at 5-HT_{1A} receptor:

Membranes of CHO cells stably expressing recombinant human 5-HT_{1A} receptor were obtained from NEN (catalog no. CRM035, GenBank no. X13556). The membranes were stored at -70 °C. Prior to use, membranes were thawed and rehomogenized in assay buffer ($MgCl_2$, NaCl, and EDTA in Tris-HCl, pH 7.4). Membranes (~ 10 µg of protein) were incubated at 37 °C for 30 min (shaking water bath) in duplicate in a total volume of 800 µL of buffer containing $MgCl_2$ (3 mM), NaCl (120 mM), EDTA (0.2 mM), GDP (10 µM), [35^S]GTPgammaS (0.1 nM), Tris (50 mM), and test compounds (compounds of the invention). Prior to addition to the incubation mixture, the test compounds were dissolved in twice distilled water. DMSO was used to aid in solubilizing certain compounds. Nonspecific binding was defined with 0.1 µM GTPgammaS.

5-HT was tested as standard in each experiment at concentrations of 100, 30, and 10 nM. Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Subsequently, the filters were rinsed twice with 5 mL of ice-

cold Tris-HCl and placed in scintillation vials. Radioactivity was extracted in 4 mL of scintillation fluid (Ultima Gold, Packard Instruments, Frankfurt, Germany) and determined by liquid scintillation counting. Binding isotherms were analyzed by nonlinear regression. Agonist efficacy (= E_{max}) is expressed relative to that of 5-HT (= 100%), which was tested at a maximally active concentration (0.1 mM) in each experiment. EC_{50} values were defined as the concentration of the compound at which 50% of its own maximal stimulation was obtained. Detailed results are depicted in **Table 1** below:

Table 1

Compound	E_{max}	EC_{50} [nM]
N-(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-acetamide	89%	4.3
3-{4-[4-(3-Amino-2-oxo-2H-chromen-6-yl)-piperazin-1-yl]-butyl}-1H-indole-5-carbonitrile	93%	2.8
(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-carbamic acid ethyl ester	89%	14.0
N-(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-2,2-dimethyl-propionamide	87%	30.0
(6-{4-[4-(5-Cyano-1H-indol-3-yl)-butyl]-piperazin-1-yl}-2-oxo-2H-chromen-3-yl)-carbamic acid methyl ester	86%	6.1

The E_{max} values determined by this assay reveal that the tested chromenonindole derivatives of the invention act in a 5-HT like fashion and hence are potent and effective 5-HT_{1A} receptor agonists. These experimental data already impressively demonstrate that the instant invention is sufficiently enabled with regard to treating the described specific human diseases.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that wilful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Jan 07, 2009
Date

C. van Amsterdam
Christoph van Amsterdam